STUDIES ON PROPAGATION OF MICROBES IN THE AIRBORNE STATE

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INTRODUCTION

The third quarterly progress report (a) reiterated the rationale for attempting to determine whether microbes are able, under any circumstance, to propagate in the airborne state, (b) outlined the experimental approach and (c) defined the continuity of investigative areas. The document included a report that airborne microbes (a) can sustain metabolic function, (b) are able to produce at least one new generation, and (c) appeared to incorporate small amounts of labelled DNA base material into the DNA (acid insoluble material).

It was noted that higher levels of incorporation of the label into DNA must be demonstrated if new DNA production were to be accepted as fact, and that different, corroborative proof that DNA could be produced was also required. The best proof is, obviously, to find a situation where the number of airborne cells increases more than 2-fold. However, if phage is produced by cells in the airborne state, then new DNA is being formed. However, the conservative viewpoint might hold that this does not constitute proof of transcription of the specific DNA needed by the microbe to continue the process of propagation.

We have, therefore, conducted experiments intended to increase the sensitivity of the measurement of DNA formation, both by the use of labelled base and by the use of a phage-sensitive species of ubiquitous microbe.

EXPERIMENTAL DETAILS AND RESULTS

A. Incorporation Studies

Details of the growth of the test bacteria, (Serratia marcescens, SM) the formation, mixing and containment of aerosols, and the techniques for incorporating and measuring 3H-thymidine have been previously reported.

Aerosols of the test bacteria (8-hr culture) and of ^{3}H-thymidine, both of which were suspended in tryptone broth plus glycerin and 2-deoxy adenosine, were mixed in air prior to filling the 1500 l aerosol chamber. A control experiment run simultaneously and in an identical chamber utilized the same bacterial suspension, but cells were killed with 1% formalin.

A massive sample was then taken after a selected interval of aerosol time. Five impingers containing 20 ml of 1% formalin were operated in parallel for 20 minutes, sampling 1250 l of air. Each

impinger lost about 3 ml of fluid during the sampling period, so the total volume of collection fluid was 85 ml. Samples of the fluid were counted for tritium content, the fluid was centrifuged, poured off and recounted. Material retained in the centrifuge tubes (cells) was resuspended in 3 ml of the formalin solution. Aliquots were removed for counting and 0.6 ml of 50% trichloroacetic acid (TC) was added. After 10 minutes the solution was filtered according to the procedure of Straat (1974) and the radioactivity on the filter pad was counted.

Results are shown in Table 1. The important finding is the contrast between the decay per minute (dpm) in the insoluble fraction from live or dead cells in aerosols. The chi-square score for the two sets of figures is 207; (a score of 5 or greater represents a highly significant difference), so there is no doubt that live cells are incorporating thymidine into new DNA material.

Although the evidence in run 1-24 was, alone, convincing enough (more than 3-fold difference) we were concerned with the apparently high count in the dead-cell insoluble fraction. That is, it was possible that nonbiological interaction had occurred. Hence, the second run (2-19) was accomplished, and this time the count in the dead-cell run was essentially background. As an additional test, a simulated, in vitro, sample, using the same volume of fluid and numbers of cells but with an increased thymidine content, was run. Percent entrainment of counts in the insoluble fractions compared favorably to the percentage found in the second run (2-19), so we conclude that the high count with dead cells in run 1-24 was contamination in some inexplicable manner, and the true count should have been not more than 4% of the suspension fluid count, i.e. about 240 dpm.

B. Phage Production

A phage sensitive strain of Escherichia coli (EC) labelled B3000, and a T_3 coliphage, were obtained from the bacteriology department of the University of California. By successive transfer of the microbe in the selected, chemically-defined minimal medium used to cultivate SM, we isolated a variant that would grow well with glycerin as an energy and carbon source, NH_4^+ as a nitrogen source and having the property to produce phage at concentrations approaching 10^9/ml.

One mixed-aerosol experiment with phage has been conducted, but the data have not been fully analyzed. However, the ratio of the phage content to the bacterial content increased during the last 12 hr interval of the aerosol-time, which is indicative of phage production.

C. Discussion and Future Work

With the exception of direct proof that the test microbe can initiate more than 1 division, the evidence now available (that new DNA can be created and that new phage is apparently formed) is sufficient to leave little doubt that under ideal circumstances, and with a test organism that might be more adaptable than the one used, true propagation of a microbial species in the airborne state can occur. We believe that the additional time and effort that might be involved in finding an ideal circumstance to prove extended division in air in a direct manner will be less responsive to the central question regarding Pg in the Jovian atmosphere than obtaining information about anaerobic microbial aerosols in reducing gases. Nothing is known of the latter, and since this environment is more nearly equal to the Jovian situation than is air, we propose to conduct future biological studies with anaerobes.

We are restricted to work with particles 2 to 3 μm in diameter in order to obtain the most long lasting aerosols possible within microbial dimensions (these particles would, however, be equally persistent in the Jovian atmosphere). Such particles probably do not contain enough nutrients to support extended growth, so "feeding" the particles in either vapor or particulate form must be done if growth is to continue. Since nutritional requirements of anaerobiosis, as well as rates and mechanics of feeding, are different than for aerobic growth, the study of anaerobes approaches the primary question more directly than continued studies with aerobes.

As noted in the Third Quarterly report, if a species is to remain resident in the Jovian atmosphere, the doubling time must exceed the half-life time of removal of particles by turbulence. We will study rates of removal of particles as a result of both gravitational settling and turbulence in an aerosol chamber large enough to permit the simulation of thermal turbulence as it might occur in the unenclosed environment. The data so obtained will be compared to models of turbulence and both the biological and physical parameters concerned in simulating microbial growth in Jovian environments will be evaluated.

D. Summary of [illegible]'s Work

1. ^{14}C metabolic studies were completed and terminated.

2. In vitro incroporation studies of ^{3}H-thymidine were completed, and techniques applicable to studies of incorporation in the airborne state were evolved.

3. Studies of both mixed aerosols, and of pre-incorporated aerosols (seeking evidence of dilution of the label) were conducted. Data from the former, included in this report, show incorporation occurred.

4. During attempts to show dilution of the label, we found evidence that at least one doubling of airborne cells is possible, as shown both by an increased number of viable cells and an increased number of cell-size units measured by the Coulter Counter.

5. Presumptive evidence of phage formation in the airborne state has been obtained.

LITERATURE CITED

Straat, Patricia A. 1974. Interim Report to NBRL, Detection of growth in aerosols, Biospherics, Inc., Rockville, Md.

Table 1. Dpm[1] found in fractions of S. marcescens aerosols coagulated with aerosols of ^{3}H-thymidine

	Aerosol Exposure				In Vitro Exposure (Dead Cells)
	Run 1-24		Run 2-19		
	Live	Dead	Live	Dead	
A In fluid collected in impingers, per ml	1.06×10^4	9.60×10^4	3.71×10^4	3.50×10^4	
B In suspensions of centrifuged material	7.55×10^3	6.25×10^3	4.00×10^3	1.75×10^3	4.92×10^6, 9.18×10^5, 4.99×10^6
C In TCA[2] insoluble material on filter	2.47×10^3[3]	7.02×10^2	1.65×10^3[3]	4.2×10^1	5.70×10^3, 2.20×10^3, 1.84×10^4
% of insoluble material in the suspension fluid; C/B X 100	32.7	11.2	41.3	2.4	1.2, 2.4, 3.6

[1] Disintegrations per minute (counts)
[2] See Text.
[3] Significant contrasting values